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(71) Applicant *(for AU GB only)*: UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).

(71) Applicant *(for all designated States except AU GB US)*: UNILEVER NV [NL/NL]; Burgemeester's Jacobplein 1, NL-Rotterdam (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : CARR, Anthony, Hugh [GB/GB]; 2 Church Road, Stevington, Bedfordshire MK43 7QB (GB). MAY, Keith [GB/GB]; 33 Molivers Lane, Bromham, Bedfordshire MK43 8JT (GB). MUNDILL, Paul, Henry, Charles [GB/GB]; 4 Brookside, Stanwick, Wellingborough, Northamptonshire NN9 6PW (GB). PRIOR, Michael, Evans [GB/GB]; 330 Newton Road, Rushden, Northants NN15 0SY (GB).

(74) Agent: BUTLER, David, John; Patent Division, Unilever PLC, Unilever House, Blackfriars, London EC4P 4BQ (GB).

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(54) Title: ANALYTICAL TEST DEVICE FOR SPECIFIC BINDING ASSAYS

(57) Abstract

An analytical test device incorporates a porous membrane or filter through which an aqueous liquid must flow during the test, and a body comprising a microporous material of higher capillary suction force than the membrane or filter, in liquid-conductive contact with the membrane or filter, which acts as a "sink" to control the liquid flow path through the membrane or filter. Preferably the body comprises a clay or a molecular sieve material.

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Analytical test device for specific binding assays

The present invention relates to assays involving specific binding, especially immunoassays.

In particular, the invention relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to give an analytical result rapidly and which require the minimum degree of skill and involvement from the user. The use of such test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace.

In the specification of UK patent application GB 2204398A we describe test devices which are readily usable even by an unskilled person and which typically merely require that some portion of the device is contacted with a sample (e.g. urine in the case of a pregnancy or ovulation test) and thereafter no further actions are required by the user before an analytical result can be observed. The analytical result can be observable within a matter of minutes following sample application, e.g. ten minutes or less.

The use of reagent-impregnated filters and membranes, such as test strips, in specific binding assays, such as immunoassays, has previously been proposed. In such procedures a sample is applied to one portion of the test strip and is allowed to permeate through the strip material, sometimes with the aid of an eluting solvent such as water. In so doing, the sample progresses into or through a detection zone in the test strip wherein a specific binding reagent is immobilised. Analyte present in the sample can participate in a sandwich or competition reaction within the detection zone, with a labelled reagent which can also be incorporated in the test strip or applied thereto. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

In all assay devices which require a liquid sample to permeate through a filter or membrane, a limiting factor on the speed of the assay is the time required for the sample to progress through the device. Furthermore, if it is necessary for the assay to handle a substantial volume of sample liquid, or the assay requires the subsequent passage of a further liquid before the assay result can be determined, the speed of the assay is limited by the capacity of the device to handle such volumes of liquid rapidly.

It is an object of the present invention to speed up the assay time in such devices, and where appropriate to enable such devices to handle relatively substantial volumes of liquid more rapidly.

The volume of sample that a particular assay can handle is also a limiting factor on the sensitivity of an assay, since a large sample will obviously contain a larger volume of any analyte.

It is therfore an object of the invention to provide an assay with increased sensitivity through being able to handle a relatively large volume of sample.

To facilitate these objects the use of "sink" materials in assay devices is already known. If an assay device incorporates, for example, a strip of porous membrane material along which a sample must progress, the total capacity for liquid sample can be increased if at the distal end of the membrane or filter it contacts a body of liquid absorbent material, such as paper. Although such conventional sinks can provide an increase the total liquid capacity of the device, they do not significantly contribute to the speed with which the device provides an analytical result, nor do they substantially increase the volume of sample that can be tested by the device in a given time.

Furthermore, we have observed that if, for example, a strip of nitrocellulose membrane is contacted towards its distal end with a sink of paper or similar fibrous material which has liquid contact with the membrane at a point which is not at the extreme distal end, an aqueous sample applied to the membrane will progress entirely to the end of the membrane before any significant amount of aqueous liquid crosses into the sink material. Even if there is substantial overlap between the nitrocellulose membrane and the paper sink, the aqueous sample still prefers to saturate the entire length of the nitrocellulose membrane before entering the paper sink.

The time that the sample takes to migrate along the entire length of membrane is dictated by the membrane length, and the flow rate of the sample through the membrane decreases as the distance travelled by the sample through the membrane increases. Therefore, it can readily be seen that the time in which an assay result can be observed is heavily dependent on the length of membrane used. Because the flow effects discussed above are observed when paper sinks are used with nitrocellulose membranes, a paper sink does not reduce the running time of an assay conducted on a nitrocellulose membrane.

A further problem that we have observed with ordinary paper sinks is that after completion of an assay, when the sink may be saturated with an aqueous sample, the membrane can dehydrate, and a reverse flow of liquid from the paper sink may occur. This reverse flow can send labelled material back along the membrane or filter and may give rise to a potentially confusing assay result.

By the invention we have found that all of these problems can be mitigated if a sink comprising microporous material is used.

The invention involves the use of a body comprising microporous material as a "sink" to control the flow path of an aqueous liquid through a porous membrane or filter.

Thus, according to the invention, there is provided an analytical test device incorporating a porous membrane or filter through which an aqueous liquid must flow during the test, also incorporating a body comprising a microporous material of higher capilliary suction force than the membrane or filter, in liquid-conductive contact with the membrane or filter, which acts as a "sink" to

control the liquid flow path through the membrane or filter.

It will be readily appreciated by the man skilled in the art that the term "capilliary suction force", as used in this patent application and claims, relates to the ability of one material in physical contact with a second material to direct fluid that originated in the second material and would normally flow by capilliarity in the second material, from the second material into the first. If the first material is of higher "capilliary suction force" than the second, then the diversion of fluid from the second material into the first will be seen to occur, since the fluid will have a preference, to a greater or lesser extent, for flowing in the first material compared to the capilliary force it experiences in the second material.

Although the term "capilliary suction force" is a relative term between two given materials and may depend, for example, on the nature of the liquid sample involved (eg the presence and size of other component molecules of the sample), the relative capilliary suction force between two materials will intimately depend on the relative mean pore diameters of the two materials. Generally, the material with the smaller mean pore diameter will have the higher capilliary suction force.

Typically, and in preferred embodiments of this invention, such a membrane comprises nitrocellulose. To obtain a higher capillary suction force, the material from which the sink is made should have a finer network of capillaries or pores than the membrane.

With regard to any microporous material, it is possible to assign to the material a "mean pore diameter", measurable by mercury porosimetry. When working with such microporous materials it is possible to evaluate other properties with respect to the materials, such as the mean pore or void volume or size, the surface area of the material, or the mean pore diameter. However, within the scope of the invention, all of these parameters would be understood by the man skilled in the art to relate to similar and interrelated physical properties for enabling the invention to be put into practice.

To provide a good sink material, it is desirable that the microporous material from which the sink is made has a mean pore diameter of less than the mean pore diameter of the membrane; preferably less than 50% of the mean pore diameter, more preferable less than 10%, more preferable less than 5%, more preferable less than 2%. As a general rule, decreasing the mean pore diameter of the sink as much as possible will generate a higher capillary suction force.

Although the figures quoted above are preferred values for the mean pore diameter of the sink material, the actual values may depend on distribution of pore diameters about the mean pore diameters of the sink and membrane. If the measured pore diameters of each material vary largely about the mean value, it may be desirable to have a large difference (say a factor of ten or more) between the mean pore diameters of both materials, to provide as small an overlap as possible between the pore sizes of the two materials, and therefore a higher capillary suction force. Conversely, if each material has a very narrow distribution of pore diameters about the mean pore diameter, it will not be necessary to have such

a large difference in mean pore diameters for the membrane and sink materials, since the overlap in pore size between them will be very small.

A preferred sink material will have not only a small mean pore diameter with respect to the membrane, but will have a reasonably large void volume, thereby allowing it to hold a reasonably large volume of fluid. In practice a sink material should have a void volume of at least 30%, more preferably around 70-80%. Also, the pores and voids in the sink material should have a high degree of interconnection.

Concerning the chemical properties of a preferred sink material, it is highly desirable that the surface area of the sink material be hydophilic, thereby allowing it to "wet" easily. It is desirable that the sink be shaped so as to have the maximum possible area of contact between the sink and the membrane or filter for optimal fluid transfer. To this end, although a preferred shape for a sink to have at least one flat surface for example in the form of a tablet, although the actual shape of the sink may depend on the nature of the sink materials. For some fine particulate sink materials (eg molecular sieve or alumina) it may not be readily possible to make the sink in the form of a tablet, although other materials do readily compress or compact into tablet form by techniques known in the art.

Regarding suitable sink materials, microporous organic polymer materials have been used. However, such organic polymers have a tendency to be hydrophobic, and therefore not "wet" easily. It is possible to overcome this by treating the polymer material with a wetting agent, for example a surfactant in a water/ethanol

mixture, and thereafter dry the polymer. Any surfactant would be suitable as the wetting agent, although preferably the surfactant is a solid at room temperature, since liquid surfactants used to treat a microporous polymer may flow on storage, thereby producing a sink which does not wet uniformly. Other suitable wetting agents include protein solutions, for example solutions of BSA. A suitable polymer material may be any polymer material which can be made into the appropriate microporous physical structure, for example ACCUREL (microporous polyprepylene), or polystyrene.

It has also been found that microporous inorganic materials may be highly suitable as sink materials. Such materials may include bentonites, clays, silicas, polyhipes, aluminas, porous glasses, silicates, ceramics, micorporous titanium, or molecular sieves. Suitable materials again have a microporous structure which is sufficiently fine to generate a sufficient capillary suction force to draw liquid from the membrane material used. Chemically, such inorganics tend to wet well, although their wetting ability can be enhanced by for example surfactant treatment.

An occasional problem with inorganic materials as sinks is that it can sometimes be difficult to manufacture them in "sheet" form. This can be overcome by pelletising the materials by techniques known in the art, such as for example by sintering, or by combining them with a small amount of binder (often a polymer) before forming the pellet. Other materials form satisfactory pellets simply by wetting them and compressing them to the desired shape and size. It can also be difficult sometimes to manufacture sinks from certain inorganic materials with

the desired physical properties (eg void volume) as they may become very brittle and fragile.

Suitable inorganic materials comprise for example molecular sieve materials, which typically have pore sizes in the region of 1-2 nanometers; such molecular sieves for example comprise synthetic zeolites, such as alumina silicates. Such zeolites (ie those structures with the capability to reversably bind water) are preferred inorganic materials. However, one disadvantage of molecular sieve materials is that the have a tendency to be able to molecularly hold (ie bind within their structure) relatively small volumes of liquid. Much of the liquid that is held in molecular sieve materials is interstitially bound between individual particles of material which have been formed together to make the pellet.

It is also desirable that the material from which the sink is manufactured remains rigid when wet.

Other possible sink materials include hydrogels (crosslinked hydrophilic polymers which can soak up water). These materials are typically found in powder or film-form when dry, but form a soft gel when wet. Whilst in principle these are highly desirable materials to use as a sink, in practice they tend not to have a sufficiently high capillary suction force to draw water out of eg nitrocellulose. Also, they tend not to hydrate sufficiently quickly for the intended usages herein, and would therefore be a non-preferred solution.

An alternative embodiment of the invention employs activated carbon paper as a sink. This has the advantage of being cheap, and it is also relatively easy to attain

the desired size and shape of sink. The activated carbon paper can be cut or folded to the desired shape or thickness of sink. The sink could comprise several pieces of activated carbon paper on top of each other.

The choice of sink material may depend on the constituency of and components in the fluid being analysed. For example, if the assay is performed using a sample of blood serum, it has been found that if moleculer seive is used as the sink, then the sink rapidly clogs up with the many relatively large protein molecules that are found in blood. However, less of a problem is experienced when an assay is conducted using urine, since this contains less protein molecules.

When analysing serum samples (or any other sample in which the presence of many large molecules may cause the sink to clog up) it has been found desirable to use a sink material of relatively large pore size, eg 100 nm or so. Such a pore size allows protein molecules to pass, but is still smaller than the pore size of most commonly used membranes (eg nitrocellulose). However, the choice of relative pore size of both the sink and the membrane will depend on, amongst other things, the nature of the sample to be analysed.

It has been found that both bentonite and charcoal paper are particularly useful sink materials for analysing both serum and urine samples. However, known techniques could be used to treat sink materials (eg molecular sieve) which are unsuitable for use with serum samples, to make them more suitable for such use.

Additionally, it has been found that molecular sieve material, if used in free particulate form (rather than

compressed into a pellet) or in a paper matrix, forms a particularly good sink material for use with both serum and urine samples. It is thought that molecular sieve materials in free particulate form (including if bound in a paper matrix) work well with serum because the serum molecules do not clog up the pores in the molecular sieve; instead the capilliary suction force of the sink is generated at least partially by the microscopic interstitial gaps between molecular sieve particles. These interstitial gaps are not so readily available if the molecular sieve is compressed into pellet form.

Nitrocellulose may also be used as a sink material with certain forms of membrane, although problems have been experienced handling non reinforced nitrocellulose, due primarily to the static inherently generated by nitrocellulose, and also its delicate nature. Also, because nitrocellulose itself is commonly found as a membrane, and a common problem with membranes is that they have a low capacity for liquid uptake, several layers of nitrocellulose on top of each other are needed if nitrocellulose itself is used as a sink.

Typically, a sink could have dimensions of 8 x 10 x 2 mm for use in the embodiments envisaged.

When utilized for example in readily available rapid assay test kits, commonly used for example in pregnancy testing kits, the use of microporous sinks having a higher capilliary suction force than the adjacent membrane has the following advantages:

a) An aqueous sample migrating through the membrane will prefer to enter the sink rather than continue in the membrane, or at least have an equal preference for

entering the sink as migrating along the membrane. Thus, the sink can be used to divert the flow along the membrane into the sink, and by appropriate placement on the membrane the working length of the membrane can be substantially reduced. This can significantly reduce the total assay time. In particular, we have noted that with nitrocellulose membranes, an aqueous sample will migrate rapidly through an initial length of the membrane, but the rate of migration progressively slows as the sample gets further and further away from its point of application to the membrane. It is therefore advantageous for the working length of the membrane to be as short as possible.

b) If the rapid assay test employs a labelled reagent which migrates along a membrane with a sample, and the labelled reagent comprises a particulate label such as latex, this reagent tends to "precipitate" at the interface between the membrane and the sink. The labelled component is therefore fixed at this interface, and even if subsequently a reverse flow of liquid did occur from the sink back into the membrane, there is much less risk that the labelled component will be carried in the reverse direction.

c) The sink may cause an increase of fluid capacity that can be dealt with in a given time by a given size of membrane or assay kit for a given assay, since a larger amount of fluid can be drawn through the membrane and stored in the sink without detrimentally effecting the chemistry occuring on the assay membrane. Therefore, this would permit the analysis by a given test of a larger liquid sample, and

therefore provide an increase in sensitivity of the assay within a given time for a given test kit.

The present invention provides, in one embodiment, an analytical test device incorporating a dry porous carrier to which a liquid sample suspected of containing an analyte can be applied, the device also incorporating a labelled specific binding reagent which is freely mobile in the porous carrier when in the moist state, and an unlabelled specific binding reagent which is permanently immobilised in a detection zone on the carrier material, the labelled and unlabelled specific binding reagents being capable of participating in either a sandwich reaction or a competition reaction in the presence of the analyte, in which prior to the application to the device of a liquid sample suspected of containing the analyte, the labelled specific binding reagent is retained in the dry state either in a zone on the carrier material upstream from the detection zone or in a macroporous body through which the applied liquid sample must pass en route to the porous carrier material, the labelled specific binding reagent being in either case freely soluble or dispersible in any liquid sample which enters the zone or macroporous body, the device incorporating a "sink" in accordance with the invention in liquid conductive contact with the porous carrier downstream from the detection zone.

Preferably, the dry porous carrier and "sink" are contained within a casing, constructed for example from moisture impervious material such as plastics material, having an access point to which the liquid sample can be applied. The detection zone should be visible from outside the casing. Preferably, the sample access point is protected by a removable cap or shroud, the cap or

shroud preferably being replaceable after the sample has been applied.

Preferably, the dry porous carrier material comprises a chromatographic membrane or filter, such as a membrane or filter of nitrocellulose. If desired, the nitrocellulose can be backed with moisture impermeable material, such as polyester sheet. Using nitrocellulose as the porous carrier material has considerable advantage over more conventional membrane or filter materials, such as paper, because nitrocellulose has a natural ability to bind proteins without requiring prior sensitisation. Specific binding reagents, such as immunoglobulins, can be applied directly to nitrocellulose and immobilised thereon. No chemical treatment is required which might interfere with the essential specific binding activity of the reagent. Unused binding sites on the nitrocellulose can thereafter be blocked using simple materials, such as polyvinylalcohol. Moreover, nitrocellulose is readily available in a range of pore sizes and this facilitates the selection of a carrier material to suit particularly requirements such as sample flow rate. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. A preferred nitrocellulose pore range is 3-8 microns.

In a preferred embodiment of the invention, the labelled specific binding reagent comprises a specific binding reagent attached to a particulate label. Such "direct labels", e.g. coloured latex particles, gold sols, non-metallic colloids, and dye sols, are already known per se. They can be used to produce an instant analytical result without the need to add further reagents in order to develop a detectable signal. They are robust and

stable and can therefore be used readily in a analytical device which is stored in the dry state. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes. Preferably, the particulate label is a latex particle, such as a coloured latex particle which can be readily visible to the eye if it becomes bound in the detection zone. If desired, the assay result can be read instrumentally, eg. by colour reflectance. Alternatively, the latex particle can incorporate a fluorescent compound which can respond to applied electromagnetic energy such as ultraviolet light or visible light, to provide an emitted signal that can be measured instrumentally. In a particularly preferred embodiment, the direct label is a coloured latex particle of spherical or near-spherical shape and having a maximum diameter of not greater than about 0.5 micron. An ideal size range for such particles is from about 0.05 to about 0.5 microns.

The optional use of a macroporous body as the portion of the device wherein the applied liquid sample encounters the particulate label considerably facilitates the ease with which the particulate label is taken up by the liquid sample, compared to the situation that usually prevails if the particulate label is incorporated as a pre-dosed reagent on the dry porous carrier membrane or filter. To enable the particulate label to migrate freely out of the macroporous body with the liquid sample, the macroporous body preferably has a pore size at least 10 times greater than the maximum particle size of the particulate label. More preferably, the macroporous body comprises plastics material having an average pore size of not less than 10 microns, and ideally about 100 microns, because such larger pore sizes give better release of the labelled reagent. The plastics material should not be

protein-binding, or should be easily blockable by means of reagents such as BSA or PVA, to minimise non-specific binding and to facilitate free movement of the labelled reagent after the macroporous body has become moistened with the liquid sample. The plastics material can be pre-treated with surface active agent or solvent, if necessary, to render it more hydrophilic and to promote rapid uptake of the liquid sample. Alternatively, if desired, a surface active agent can be incorporated in the solution containing the labelled reagent when this is applied to the macroporous material during manufacture of the device.

The labelled reagent is preferably incorporated in the macroporous material in bulk, eg. large sheet, form before it is subdivided into individual bodies for use in a testing device of the invention. After a solution containing the labelled reagent has been allowed to saturate the macroporous material, the macroporous material should be dried, eg. by vacuum or air-drying, or preferably by freeze-drying. Optionally, the solution can also contain a surface active agent, such as a detergent, and/or a glazing material, such as a sugar, e.g. sucrose. The presence of the glazing material appears to enhance release of the labelled reagent and promotes stability of delicate specific binding reagents such as antibodies.

Ideally, if present, the macroporous body is in direct moisture-conductive contact with the porous material, and the detection zone on the porous carrier material is spaced away from the region of contact between the porous carrier material and the macroporous body. In such an embodiment, the quantity of liquid sample required to saturate the macroporous body is preferably not less than the quantity of liquid sample capable of being

absorbed by the mass of porous carrier material linking the macroporous body and the detection zone. In other words, the liquid capacity of the macroporous body is at least equal to the liquid capacity of the working portion of the porous carrier.

The invention also provides an analytical method in which a device as set forth above is contacted with an aqueous liquid sample suspected of containing the analyte, such that the sample permeates by capillary action via the macroporous body through the porous solid carrier into the detection zone and the labelled reagent migrates therewith to the detection zone, the presence of analyte in the sample being determined by observing the extent (if any) to which the labelled reagent becomes bound in the detection zone.

In one embodiment of the invention, the labelled reagent is a specific binding partner for the analyte. The labelled reagent, the analyte (if present) and the immobilised unlabelled specific binding reagent cooperate together in a "sandwich" reaction. This results in the labelled reagent being bound in the detection zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

In another embodiment of the invention, the labelled reagent is either the analyte itself which has been conjugated with a label, or is an analyte analogue, ie a chemical entity having the identical specific binding characteristics as the analyte, and which similarly has been conjugated with a label. In the latter case, it is preferable that the properties of the analyte analogue which influence its solubility or dispersibility in an

aqueous liquid sample and its ability to migrate through the moist porous solid phase material should be identical to those of the analyte itself, or at least very closely similar. In this second embodiment, the labelled analyte or analyte analogue will migrate through the porous carrier into the detection zone and bind with the immobilised reagent. Any analyte present in the sample will compete with the labelled reagent in this binding reaction. Such competition will result in a reduction in the amount of labelled reagent binding in the detection zone, and a consequent decrease in the intensity of the signal observed in the detection zone in comparison with the signal that is observed in the absence of analyte in the sample.

In a further alternative embodiment, an analyte or analyte analogue is immobilised in the detection zone, and the labelled reagent is specific for the analyte. If an analyte-containing sample is applied to the device, competition between the immobilised and free analyte reduced the extent to which the labelled reagent may become bound in the detection zone.

In a further embodiment of the present invention, the porous carrier is linked, via the macro-porous body if present, to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous carrier. Preferably, the porous carrier (and the macroporous body) are contained within a moisture-impermeable casing or housing and the porous receiving member extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and reach the porous carrier. The housing should be provided with means, e.g. appropriately placed apertures, which enable the detection zone of the porous

solid phase carrier material (carrying the immobilised unlabelled specific binding reagent) to be observable from outside the housing so that the result of the assay can be observed. If desired, the housing may also be provided with further means which enable a further zone of the porous solid phase carrier material to be observed from outside the housing and which further zone incorporates one or more control reagents which enable an indication to be given as to whether the assay procedure has been completed. Preferably the housing is provided with a removable cap or shroud which can protect the protruding porous receiving member during storage before use. If desired, the cap or shroud can be replaced over the protruding porous receiving member, after sample application, while the assay procedure is being performed.

An important embodiment of the invention is a pregnancy testing device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing, the porous nitrocellulose carrier and the sample receiving member optionally being linked via a macroporous body such that any sample reaching the porous carrier must first pass through the macroporous body, the sample receiving member (and the macroporous body) acting as a reservoir from which urine is released into the porous carrier, the device containing pre-dosed in a zone of the porous carrier, or in the macroporous body if present, a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a detection zone on the carrier spatially distant from the pre-dosed zone or the macroporous body a highly-specific unlabelled anti-hCG

antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member. A fertile period prediction device, essentially as just defined except that the analyte is LH, is an important alternative.

Such devices can be provided as kits suitable for home use, comprising a plurality (e.g. two) of devices individually wrapped in moisture impervious wrapping and packaged together with appropriate instructions to the user.

The porous sample receiving member can be made from any bibulous, porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be unidirectional (ie with pores or fibres running wholly or predominantly parallel to an axis of the member) or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight), polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile and polytetrafluoro-ethylene can be used. It can be advantageous to pre-treat the member with a surface-active agent during manufacture, as this can reduce any inherent hydrophobicity in the member and therefore enhance its ability to take up and deliver a moist sample rapidly and efficiently. Porous sample receiving members can also be made from paper or other cellulosic materials, such as

nitro-cellulose. Materials that are now used in the nibs of so-called fibre tipped pens are particularly suitable and such materials can be shaped or extruded in a variety of lengths and cross-sections appropriate in the context of the invention. Preferably the material comprising the porous receiving member should be chosen such that the porous member can be saturated with aqueous liquid within a matter of seconds. Preferably the material remains robust when moist, and for this reason paper and similar materials are less preferred in any embodiment wherein the porous receiving member protrudes from a housing. The liquid must thereafter permeate freely from the porous sample receiving member into the macroporous body.

If present, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with an antibody that will bind to the labelled reagent, e.g. an "anti-mouse" antibody if the labelled reagent is an antibody that has been derived using a murine hybridoma, to confirm that the sample has permeated the test membrane or filter. Alternatively, the control zone can contain an anhydrous reagent that, when moistened, produces a colour change or colour formation, e.g. anhydrous copper sulphate which will turn blue when moistened by an aqueous sample. As a further alternative, a control zone could contain immobilised analyte which will react with excess labelled reagent from the first zone. As the purpose of the control zone is to indicate to the user that the test has been completed, the control zone should be located downstream from the detection zone in which the desired test result is recorded. A positive control indicator therefore tells the user that the sample has permeated the required distance through the test device.

The label can be any entity the presence of which can be readily detected. Preferably the label is a direct label, ie. an entity which, in its natural state, is readily visible either to the naked eye, or with the aid of an optical filter and/or applied stimulation, e.g. UV light to promote fluorescence. For example, minute coloured particles, such as dye sols, metallic sols (e.g. gold), and coloured latex particles, are very suitable. Of these options, coloured latex particles are most preferred. Concentration of the label into a small zone or volume should give rise to a readily detectable signal, e.g. a strongly-coloured area. This can be evaluated by eye, or by instruments if desired.

Indirect labels, such as enzymes, e.g. alkaline phosphatase and horse radish peroxidase, can be used but these usually require the addition of one or more developing reagents such as substrates before a visible signal can be detected. Hence these are less preferred. Such additional reagents can be incorporated in the porous solid phase material or in the macroporous body, or in the sample receiving member if present, such that they dissolve or disperse in the aqueous liquid sample. Alternatively, the developing reagents can be added to the sample before contact with the porous material or the porous material can be exposed to the developing reagents after the binding reaction has taken place.

Coupling of the label to the specific binding reagent can be by covalent bonding, if desired, or by hydrophobic bonding. Such techniques are commonplace in the art, and form no part of the present invention. In the preferred embodiment, where the label is a direct label such as a coloured latex particle, hydrophobic bonding is preferred.

In certain embodiments of the invention, the labelled reagent may migrate with the liquid sample as this progresses to the detection zone. The flow of sample should continue beyond the detection zone and sufficient sample should be applied to the porous carrier material in order that this may occur and that any excess labelled reagent which does not participate in any binding reaction in the detection zone is flushed away from the detection zone by this continuing flow.

The presence or intensity of the signal from the label which becomes bound in the detection zone can provide a qualitative or quantitative measurement of analyte in the sample. A plurality of detection zones arranged in series on the porous solid phase material, through which the aqueous liquid sample can pass progressively, can also be used to provide a quantitative measurement of the analyte, or can be loaded individually with different specific binding agents to provide a multi-analyte test.

The immobilised reagent in the detection zone is preferably a highly specific antibody, and more preferably a monoclonal antibody. In the embodiment of the invention involving the sandwich reaction, the labelled reagent is also preferably a highly specific antibody, and more preferably a monoclonal antibody.

Preferably the porous carrier material is in the form of a membrane or filter or sheet to which during manufacture of the device, one or more reagents can be applied in spacially distinct zones. During use, the liquid sample is allowed to permeate through the sheet or membrane or filter from one side or end to another.

If desired, a device according to the invention can incorporate two or more discrete bodies of porous solid phase carrier material, e.g. separate membrane or filters or sheets, each carrying immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device initiates sample flow in the discrete bodies simultaneously. The separate analytical results that can be determined in this way can be used as control results, or if different reagents are used on the different carriers, the simultaneous determination of a plurality of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that proteinaceous reagents, such as an antibody, in the detection zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of an antibody in the second zone needs to be performed by chemical coupling using, for example, CNBr, carbonyldiimidazole, or tresyl chloride.

Following the application of the specific binding reagent to the detection zone, the remainder of the porous solid phase material should be treated to block any remaining binding sites elsewhere. Blocking can be achieved by treatment with protein (e.g. bovine serum albumin or milk protein), or with polyvinylalcohol or ethanolamine, or any combination of these agents, for example. Between these process steps the porous solid phase carrier material should be dried.

Preferably, the nitrocellulose sheet is "backed", e.g. with plastics sheet, to increase its handling strength. This can be manufactured easily by forming a thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose can be tightly sandwiched between two supporting sheets of solid material, e.g. plastics sheets.

It is preferable that the flow rate of an aqueous sample through the porous solid phase material should be such that in the untreated material, aqueous liquid migrates at a rate of 1cm in not more than 2 minutes, but slower flow rates can be used if desired. However, an advantage of the present invention is that it does facilitate higher flow rates of liquid through the porous solid phase material. The spatial separation between the macroporous body and the detection zone, and the flow rate characteristics of the porous carrier material, can be selected to allow adequate reaction times during which the necessary specific binding can occur.

Preferably, the immobilised reagent in the detection zone is impregnated throughout the thickness of the carrier in the detection zone (e.g. throughout the thickness of the sheet or membrane or filter if the carrier is in this form). Such impregnation can enhance the extent to which the immobilised reagent can capture any analyte or labelled reagent, present in the migrating sample.

Reagents can be applied to the porous carrier material in a variety of ways. Various "printing" techniques have previously been proposed for application of liquid reagents to carriers, e.g. micro-syringes, pens using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the present context. To facilitate manufacture, the carrier (e.g. sheet) can be treated with the reagents and then subdivided into smaller portions (e.g. small narrow membrane or filters each embodying the required reagent-containing zones) to provide a plurality of identical carrier units.

An assay based on the above principles can be used to determine a wide variety of analytes by choice of appropriate specific binding reagents. The analytes can be, for example, proteins, haptens, immunoglobulins, hormones, polynucleotides, steroids, drugs, infectious disease agents (e.g. of bacterial or viral origin) such as Streptoccus, Neisseria and Chlamydia. Sandwich assays, for example, may be performed for analytes such as hCG, LH, and infectious disease agents, whereas competition assays, for example, may be carried out for analytes such as E-3-G and P-3-G.

The determination of the presence (if any) of more than one analyte in sample can have significant clinical utility. For example, the ratio of the levels of apolipoproteins A_1 and B can be indicative of susceptibility to coronary heart disease. Similarly, the ratio of the levels of glycated haemoglobin (HbA) to unglycated (HbAo) or total (Hb) haemoglobin can aid in the management of diabetes. Additionally it is possible to configure tests to measure two steroids simultaneously, e.g E-3-G and P-3-G.

The determination of the presence of more than two (ie multiple) analytes in any sample may have significant clinical utility. For example, the detection of the presence of various different sereotypes of one bacterium, or the detection of the presence of soluble serological markers in humans may be useful. By way of example, a multiple analyte test for the detection of the presence of different serotypes of Streptococcus can be prepared for groups A, B, C and D. A cocktail of monoclonal antibodies, each specific for various pathologically important group serotypes, or a polyclonal antiserum raised against a particular Streptococcal group, can be dispensed onto a porous carrier membrane or filter as a line extending the width of the membrane or filter of approximately 1mm zone length. Multiple lines be dispensed in spatially discrete zones, each zone containing immunochemically reactive component(s) capable of binding the analyte of interest. Following the application of the multiple zones, via a suitable application procedure (eg ink-jet printing, metered pump and pen, airbrush), the remainder of the porous material should be treated with a reagent (eg bovine serum albumin, polyvinylalcohol, ethanolamine) to block any remaining binding sites elsewhere.

By way of example only, some preferred embodiments of the invention will now be described in detail with reference to the accompanying drawings.

Embodiment 1

Figure 1 of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and Figure 2 represents a cross-sectional side elevation of the device shown in Figure 1.

Referring to Figure 1, the device comprises a housing or casing 100 of elongate rectangular form having at one end 101 a portion 102 of reduced cross-sectional area. A cap 103 can be fitted onto portion 102 and can abut against the shoulder 104 at end 101 of the housing. Cap 103 is shown separated from housing 100. Extending beyond end 105 of portion 102 is a porous sample collector 106. When cap 103 is fitted onto portion 102 of the housing, it covers porous sample collector 106. Upper face 107 of housing 100 incorporates two apertures 108 and 109. The housing is constructed of an upper half 110 and a lower half 111.

Referring to Figure 2, it can be seen that housing 100 is of hollow construction. Porous sample collector 106 extends into housing 100. The inner end 112 of sample collector 106 is recessed to accommodate a macroporous body 113 of plastics material. Aqueous liquid sample applied to collector 106 can pass freely into macroporous body 113, rapidly saturating it. In turn, macroporous body 113 is in liquid permeable contact with a membrane or filter of porous carrier material 114. The housing is constructed of an upper half 110 and a lower half 111 and membrane or filter 114 overlap to ensure that there is adequate contact between these two components and that a liquid sample applied to sample collector 106 can permeate via macroporous body 113 and into membrane or filter 114. Strip 114 extends further into housing 100. To help ensure that no liquid sample reaches membrane or filter 114 without first passing through macroporous body 113, a gap 115 can be left in the housing 100 by arranging for the membrane or filter 114 to overlap macroporous body 113 only partially. Strip 114 is "backed" by a supporting membrane or filter 116 formed of transparent moisture-impermeable plastics material. Strip 114 extends

beyond apertures 108 and 109. Means are provided within housing 100 by webbs 117 and 118 to hold membrane or filter 114 firmly in place. In this respect, the internal constructional details of the housing are not a significant aspect of the invention as long as the membrane or filter is held firmly in place within the housing, sample collector 106 is firmly retained in the housing, and adequate fluid permeable contact is maintained between sample collector 106, macroporous body 113 and membrane or filter 114. The transparent backing membrane or filter 116 lies between membrane or filter 114 and apertures 108 and 109 and can act as a seal against ingress of moisture from outside the housing 100 via these apertures. The device also incorporates a sink 119 comprising a tablet of compressed microporous particulate inorganic material, which is held in contact with membrane or filter 114 slightly upstream from aperture 109. Sink 119 is held in close liquid contact with membrane or filter 114 by being sandwiched between membrane or filter 114 and lower portion 111 of casing 100. If desired, the residual space 120 within the housing can contain additional moisture-absorbant material, such as silica gel, to help maintain the membrane or filter 114 in the dry state during storage. The reagent-containing detection zone in membrane or filter 114 is not depicted in Figure 2, but the zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture 108 in order that when the device has been used in an assay, the result can be observed through aperture 108. Aperture 109 provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the membrane or filter to be observed.

In operation, the protective cap 103 is removed from the holder and sample collector 106 is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing sample collector 106 to the liquid sample for a time sufficient to ensure that the collector 106 is saturated with the sample, the cap 103 can be replaced and the device placed aside by the user for an appropriate period time (e.g. two or three minutes) while the sample permeates test membrane or filter 114 to provide the analytical result. After the appropriate time, the user can observe the test membrane or filter through apertures 108 and 109 and can ascertain whether the assay has been completed by observing the control zone through aperture 109, and can ascertain the result of the assay by observing the second zone through aperture 108.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing 100 being moulded in two parts (e.g. upper and lower halves 110 and 111) which can be securely fastened together (e.g. by ultrasonic welding) after the sample collector, macroporous body test membrane or filter and sink have been placed within one of the halves and then sandwiched between the two halves. The act of forming this sandwich construction can be used to "crimp" the sample collector, macroporous body, test membrane or filter and sink together to ensure adequate contact between them. Cap 103 can be moulded as a separate complete item. If desired, apertures 108 and 109 can be provided with transparent inserts which may insure greater security against ingress of extraneous moisture from outside the housing. By providing a tight fit between the end 105 of housing 100 and the protruding sample collector 106, the application of sample to the protruding member

will not result in sample entering the device directly and by-passing collector 106. Collector 106 therefore provides the sole route of access for the sample to the membrane or filter within the housing, and can deliver sample to the membrane or filter in a controlled manner. The device as a whole therefore combines the functions of sampler and analyser.

By using the test membrane or filter materials and reagents as herein described, a device in accordance with Figures 1 and 2 can be produced which is eminently suitable for use as a pregnancy test kit or fertile period test kit for use in the home or clinic. The user merely needs to apply a urine sample to the exposed porous member and then (after optionally replacing the cap) can observe the test result through aperture 108 within a matter of a few minutes.

Although described with particular reference to pregnancy tests and fertile period tests, it will be appreciated that the device, as just described, can be used to determine the presence of a very wide variety of analytes if appropriate reagents are incorporated in the test membrane or filter. It will be further appreciated that aperture 109 is redundant and may be omitted if the test membrane or filter does not contain any control means. Further, the general shape of the housing and cap, both in terms of their length, cross-section and other physical features, can be the subject of considerable variation without departing from the spirit of the invention.

Figure 3 of the accompanying drawings shows an enlarged view of the sample collector, macroporous body

test membrane or filter and sink in the device illustrated in Figures 1 and 2.

The bibulous sample collector 106 is linked to the macroporous body 113 and test membrane or filter 114, backed by the transparent plastics sheet 116, such that liquid can flow in the direction shown by the arrows from the sample collector through the macroporous body and into the porous membrane or filter, and then into sink 119. Test zone 121 incorporates the immobilised specific binding reagent, and control zone 122 contains a reagent to indicate that the sample has permeated a sufficient distance along the test membrane or filter.

An aqueous sample deposited in collector 106 can flow into macroporous body 113 and take up labelled reagent therein. The sample can permeate from macroporous body 113 along the length of membrane or filter 114 and in so doing will carry the labelled reagent along the membrane or filter and through zones 121 and 122.

If the desired, eg. for ease of manufacture, the collector 106 need not be recessed to accommodate the macroporous body 113. Instead, these components can simply be placed in an overlapping arrangement, together with the porous membrane or filter 114, and pressed together during assembly of the complete device. This will in practice provide a physical arrangement in which the liquid path will be essentially as depicted in Figure 3.

Embodiment 2

Figures 4 and 5 illustrate another embodiment of the invention, which is seen in plan view in Figure 4 and in

cross-section in Figure 5, the cross-section being an elevation on the line A seen in Figure 4.

Referring to Figure 4, the test device comprises a flat rectangular casing 400 incorporating a centrally disposed rectangular aperture 401, adjacent the left hand end 402, and two further apertures 403 and 404 near the mid point of the device and arranged such that apertures 401, 403 and 404 lie on the central longitudinal axis of the device corresponding to line A. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in Figure 5, the device is hollow and incorporates within it a sample receiving member 405 adjacent end 402 of casing 400 and lying directly beneath aperture 401. Sample receiving member 405 is in liquid-conductive contact with one end of a test membrane or filter 406 backed by a transparent plastics sheet 407 also contained within casing 400, and which extends to the extreme other end of the casing. The transparent backing sheet 407 is in firm contact with the upper inner surface 408 of casing 400, and provides a seal against apertures 403 and 404 to prevent ingress of moisture or sample into the casing. The device also incorporates a sink 409 of microporous material which occupies substantially the entire inner volume of casing 402 downstream from aperture 404. Sink 409 is in direct contact with the distal end of membrane or filter 406 and can readily take up any aqueous liquid migrating along membrane or filter 406 as soon as the liquid has passed beyond aperture 404. Although not shown in the drawings, the porous test membrane or filter 406 incorporates a test zone and a control zone placed appropriately in relation to apertures 403 and 404, in a manner analagous to that

described in Embodiment 1. The sample receiving member can incorporate a labelled reagent which is readily soluble or dispensable in an applied liquid sample, or such reagent can be pre-dosed onto the test membrane or filter in a zone upstream from the test zone.

In operation, an aqueous sample can be applied through aperture 401, e.g. by means of a syringe, to saturate porous receiving member 405. Thereafter, the aqueous sample can permeate the test membrane or filter and, after an appropriate time, the test result can be observed through apertures 403 and 404.

Embodiment 3

Figure 6 shows a cross section of an alternative sample collector, macroporous body, test membrane and sink arrangement according to the invention. The bibulous sample collector 606 and macroporous body 613 are of the same type as previously described. Test membrane 614 comprises a transparent plastics backing sheet 616, thermoformed so as to have a moulded well 650. Contained in well 650 is an "envelope" of microporous material, for example powdered molecular sieve material 651. The molecular sieve material 651 is sealed into well 650 by two layers of nitrocellulose. Layer 652 of nitrocellulose material has a pore size of 0.1 microns, and one of its primary purposes is to help seal molecular sieve 651 in well 650. On top of this is nitrocellulose layer 653, of pore size 3 microns, in which the bulk of the sample applied will run in the direction shown by the arrows. Test membrane 614 also incorporates a test zone 621, containing immobilized specific binding reagent, and control zone 622, analagous to those described in previous embodiments.

An aqueous sample deposited in collector 606 can flow into macroporous body 613 and take up labelled reagent therein. The sample can permeate from macroporous body 613 along the length of membrane 614 and in so doing will carry the labelled reagent along the membrane and through zones 621 and 622, and then onwards towards the sink, where at least some of it enters and is retained by the molecular sieve material 651.

Embodiment 4

Figures 7 and 8 of the accompanying drawings illustrate a further test device according to the invention. Figure 7 illustrates the complete device viewed from the front, and Figure 8 depicts the same device partially cut away to reveal details of a assay arrangement contained within the body of the device.

Referring to Figure 7, the device comprises an elongate body 700 terminating at its lower end 701 in a small receptacle 702 which can hold a predetermined volume of a liquid sample, eg urine. Receptacle 702 may be manufactured integrally with body 700; alternatively it may conveniently be manufactured separately to body 700, and attached thereto at a convenient moment prior to the commencement of the assay. The front face 703 of the body 700 incorporates two square small apertures or windows 704 and 705 located one above the other.

Referring to Figure 8, the elongate portion of body 700 is hollow and incorporates a test strip 706 running along the body. Test strip 706 is made of plastics backed nitrocellulose (or alternatively filter paper), and terminates at end 707. Test strip 706 is held in place in body 700 by retention webs (not shown). Test strip 706

conveniently dips into the uring sample when an adequate sample of urine is held in receptacle 702 and the device is held vertically. Located adjacent to end 707 of strip 706 is a horizontal zone 708 (shown by dotted lines) of labelled specific binding agent that can freely migrate in the strip in the moist state. Strip 706 extends beyond apertures 704 an 705. The internal features of housing 700 are such as to try to ensure that when strip 706 is wet, it is nevertheless held firmly in place in housing 700.

In embodiments of the invention where the strip has a transparent plastics backing, conveniently the plastics backing strip lies between strip 706 and apertures 704 and 705 to act as a seal against the ingression of moisture from outside housing 700 via these apertures. Housing 700 can also contain additional desiccants such as silica gel (not shown) to help maintain the strip in a dry state during storage.

The device also has located down stream on strip 706 from apertures 704 and 705 a microporous sink 709, in liquid contact with strip 706. Sink 709 comprises a tablet of molecular sieve material. Because sink 709 has a higher capilliary suction force than strip 706, water is drawn into sink 709 in preference to flowing further though strip 706. For reasons previously discussed, this allows the length of strip 706 to be shorter than is usual for similar assays.

The reagent-containing detection zone in strip 706 is not depicted in Figure 8, but the zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture 704 in order that when the device has been used in an assay, the result can be observed

through aperture 704. Aperture 705 provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample is retained in receptacle 702. Conveniently once the sample has been introduced into receptacle 702, the device is provided with a stand to hold the device in a substantially upright position whilst the test runs. From the receptacle 702 the liquid sample rises by capillary action through the test strip 706 and conveys the labelled reagent from zone 708 to the two circular zones 704 and 705.

Depending on the presence or absence of the analyte, a specific binding reaction may occur on test strip 706 adjacent to aperture 704. If the labelled specific binding reagent contains a visible marker and the analyte was present in the liquid sample, then if the assay was of the "sandwich" format a visible signal will observable through aperture 704. Other types of label may be detected by the appropriate means.

In any event, the zone on strip 706 adjacent aperture 705 acts as a "control", and the presence of a signal observable through this aperture indicates that the test has been properly performed (irrespective of whether or not the sample contains the analyte to be determined).

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone beneath aperture 704 can contain immobilised HCG which will bind a labelled

antibody which is carried upwards from zone 708 by the migrating liquid sample. The same labelled antibody can engage in a "sandwich" reaction with hCG in the sample and be bound in on the strip adjacent aperture 704 by another specific anti-hCG antibody which has been immobilised thereon. If desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from body 708, eg an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

Embodiment 5

Figure 9 of the accompanying drawings illustrate another embodiment of the invention. The embodiment of Figure 9 is synonymous with embodiment 3, except that apertures 704 and 705 for allowing the results of the test to be observed are located on the opposite side of hollow body 700. The internal workings and structure of the device of figure 9 are the same as those indictated for figures 7 and 8.

CLAIMS

1. An analytical test device incorporating a porous membrane or filter through which an aqueous liquid must flow during the test, also incorporating a body comprising a microporous material of higher capilliary suction force than the membrane or filter, in liquid-conductive contact with the membrane or filter which acts as a "sink" to control the liquid flow path through the membrane or filter.

2. A device according to claim 1 wherein the body comprises inorganic material.

3. A device according to claim 1 or claim 2, wherein the body is in the form of a tablet of microporous material.

4. A device according to any of claims 1-3 wherein the body has a mean pore diameter less than 50% of the mean pore diameter of the membrane or filter.

5. A device according to claim 4, wherein the body level has a mean pore diameter less than 10% of that of the membrane or filter.

6. A device according to claim 4, wherein the body has a mean pore diameter less than 5% of that of the membrane or filter.

7. A device according to any of claims 1-6, wherein the inorganic material is a mineral.

8. A device according to claim 7, wherein the mineral is a clay.

9. A device according to claim 8, wherein the clay is an alumino-silicate.

10. A device according to claim 8, wherein the clay is a bentonite.

11. A device according to any of claims 2-6, wherein the body is a tablet of a molecular sieve material.

12. A device according to claim 1, wherein the body comprises a microporous organic material.

13. A device according to claim 12 wherein the microporous organic material is polymeric.

14. A device according to claim 11 or claim 12 wherein the organic material is subjected to a pretreatment with a surfactant.

15. A device according to any of the preceding claims wherein the body is essentially non swelling when wetted with an aqueous liquid.

16. A device according to any of the preceding claims wherein the porous membrane comprises nitrocellulose.

17. A device according to any of the preceding claims wherein the membrane or filter incorporates a specific binding agent immobilised in the liquid flow path upstream from the "sink".

18. A device according to claim 1 wherein the body is in the form of an envelope.

19. A device according to any one of the preceding claims, wherein the membrane or filter is a dry porous carrier to which a liquid sample suspected of containing an analyte can be applied, the device also incorporating a labelled specific binding reagent which is freely mobile in the porous carrier when in the moist state, and an unlabelled specific binding reagent which is permanently immobilised in a detection zone on the carrier material, the labelled and unlabelled specific binding reagents being capable of participating in either a sandwich reaction or a competition reaction in the presence of the analyte, in which prior to the application to the device of a liquid sample suspected of containing the analyte, the labelled specific binding reagent is retained in the dry state either in a zone on the carrier material upstream from the detection zone or in a macroporous body through which the applied liquid sample must pass en route to the porous carrier material, the labelled specific binding reagent being in either case freely soluble or dispersible in any liquid sample which enters the zone or macroporous body, the "sink" in liquid conductive contact with the porous carrier downstream from the detection zone.

20. A device according to claim 19, wherein the labelled specific binding agent comprises a specific binding agent attached to a particulate label, preferably a coloured latex particle, gold sol, non-metallic colloid or dye sol.

21. A device according to claim 20, wherein the labelled specific binding agent is contained in the dry state in a macroporous body through which the applied liquid sample must pass en route to the porous carrier.

22. A device according to any one of claims 19 to 21, wherein the porous carrier is linked, via the macroporous body if present, to a porous sample-receiving member.

23. A device according to any one of claims 19 to 22, wherein the porous carrier is contained within a casing.

24. A device according to claim 23, wherein the casing has a removable, and preferably replaceable, cap or shroud protecting an access point to which liquid sample can be applied.

25. A device according to claim 23 incorporating a sample receiving member which provides the sample access point and which protrudes from the casing.

26. Use of a microporous material as a "sink" to control the flow path of an aqueous liquid through a porous membrane or filter, the microporous "sink" having a higher capilliary suction force than the membrane or filter.

Fig. 1.

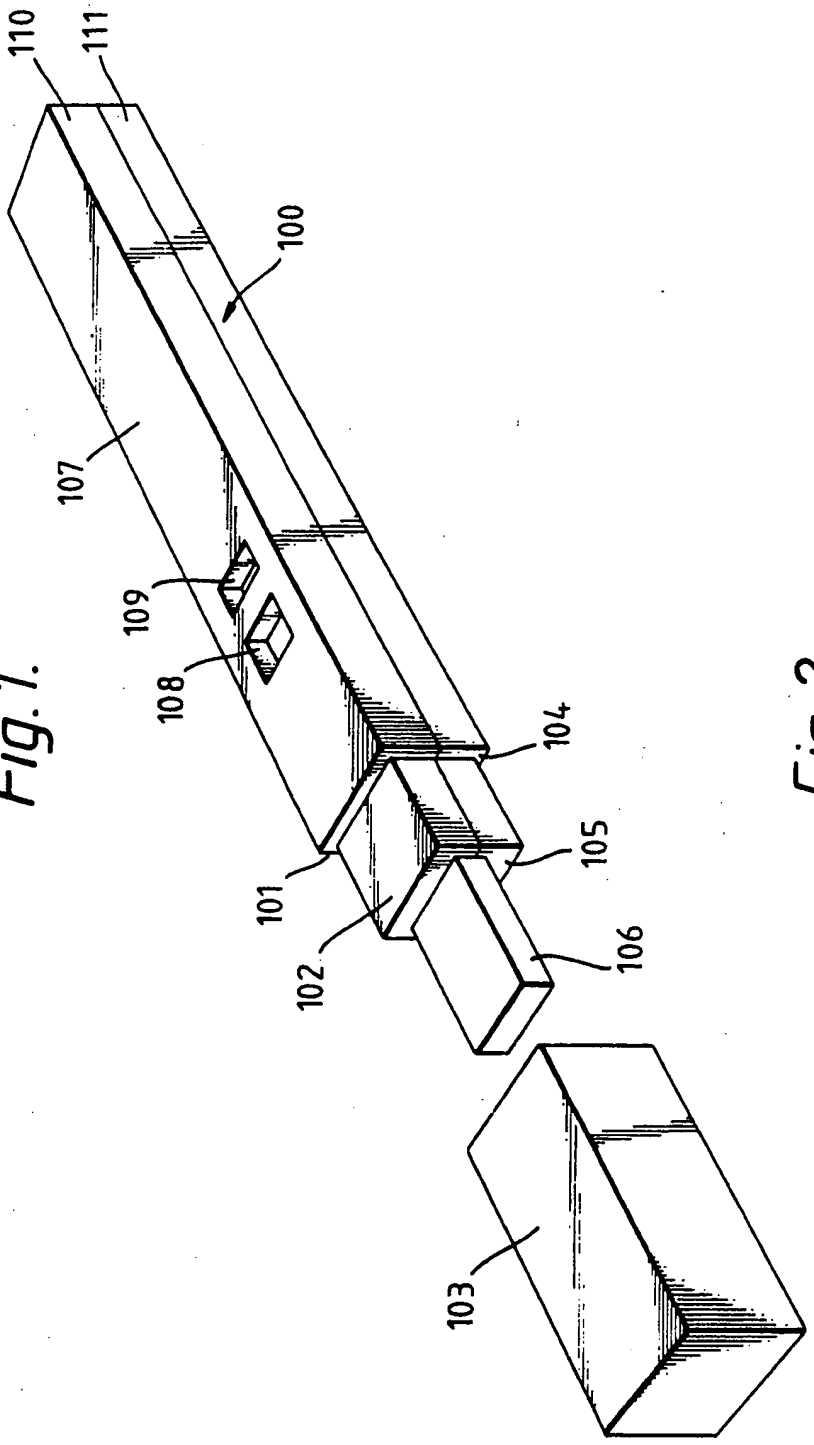
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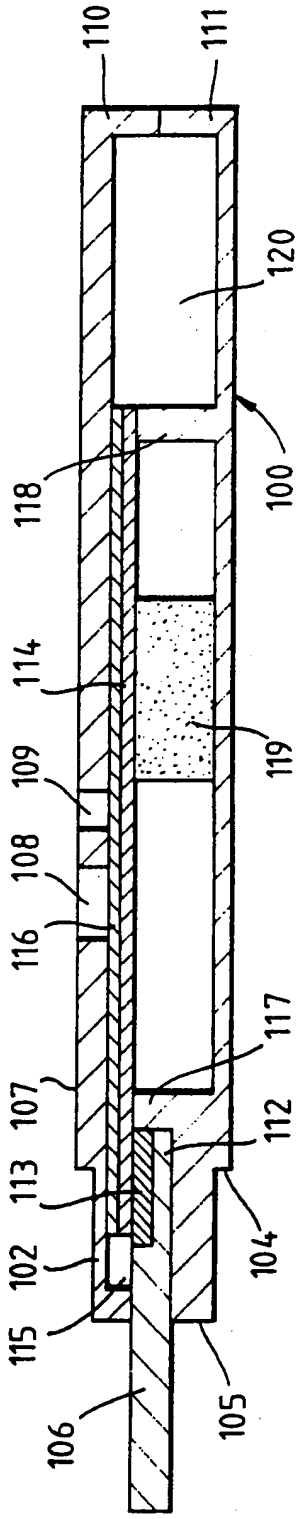
Fig. 2.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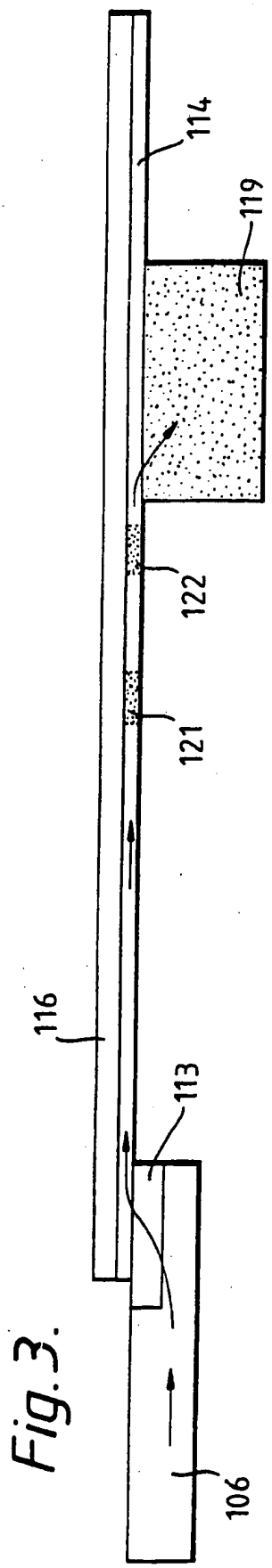


Fig. 3.

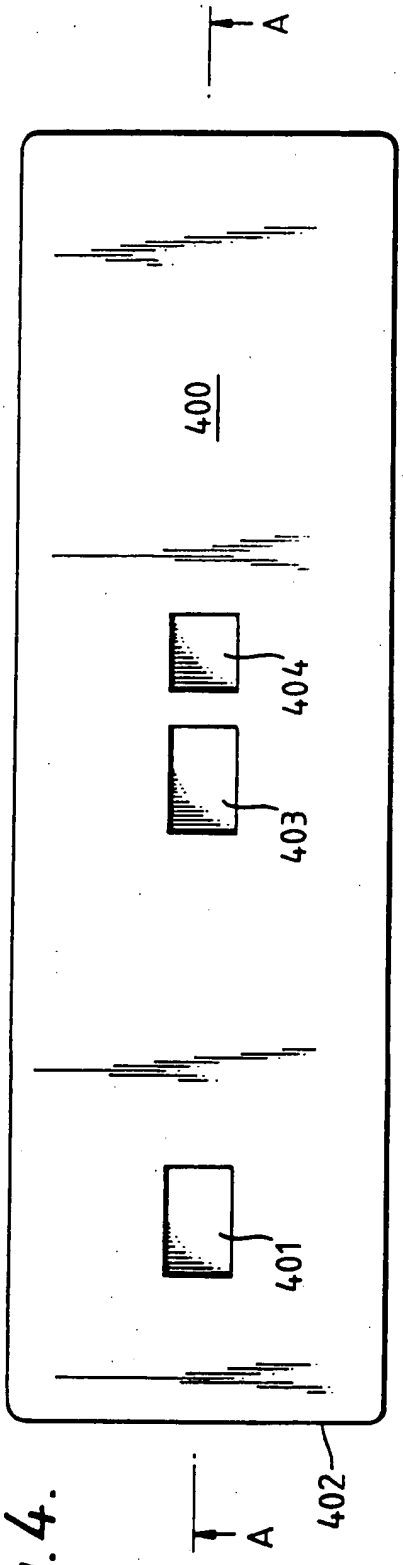


Fig. 4.

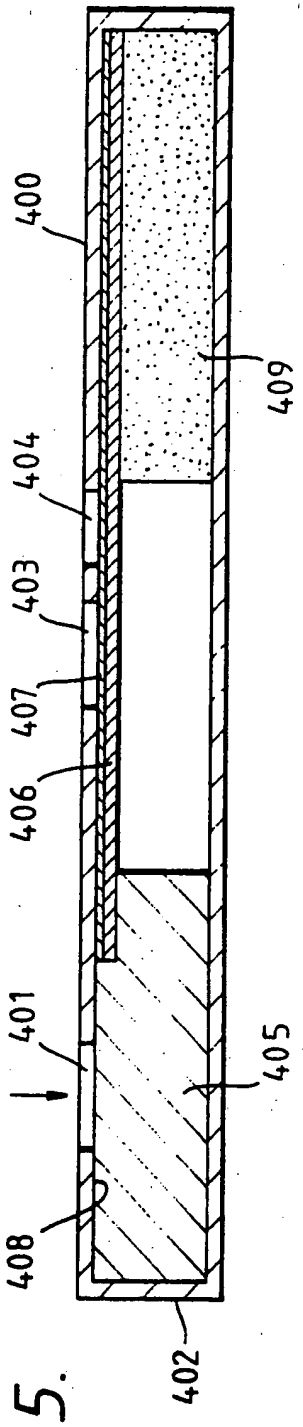


Fig. 5.

Fig. 6.

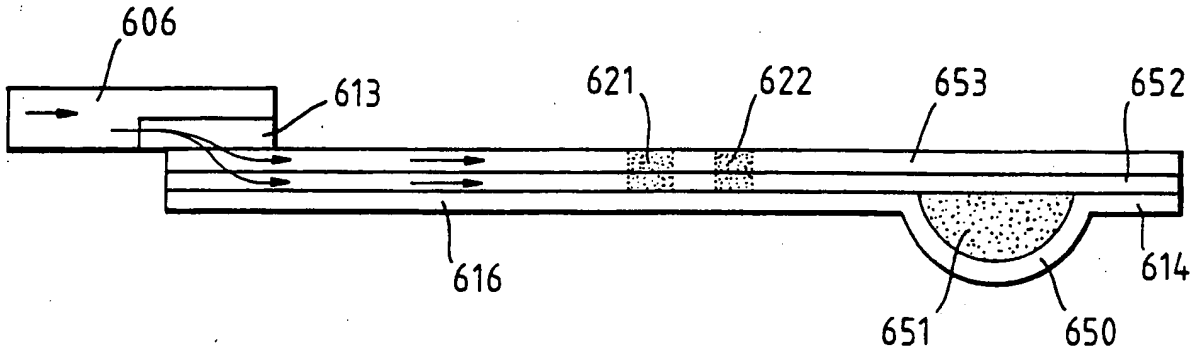


Fig. 7.

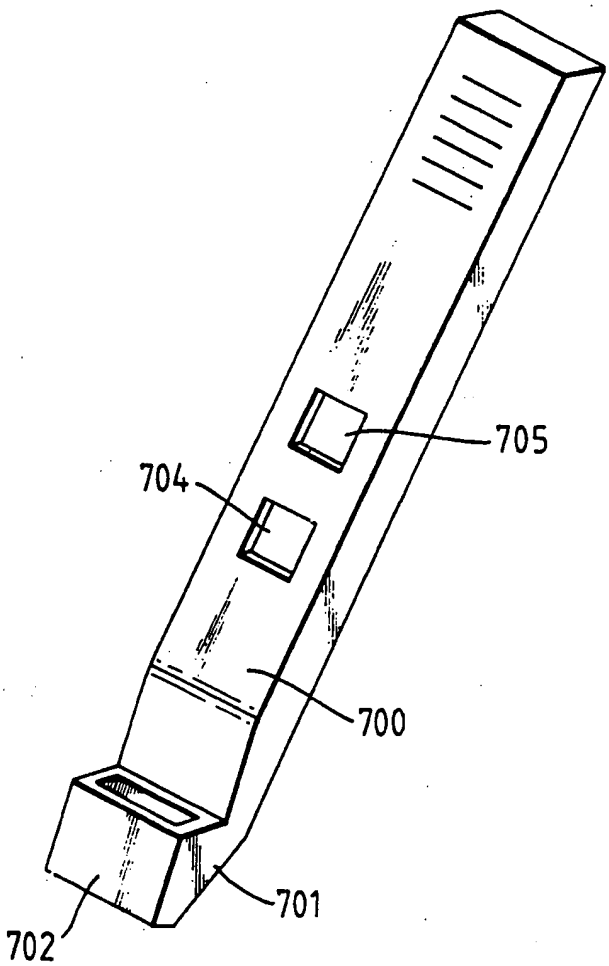


Fig.8.

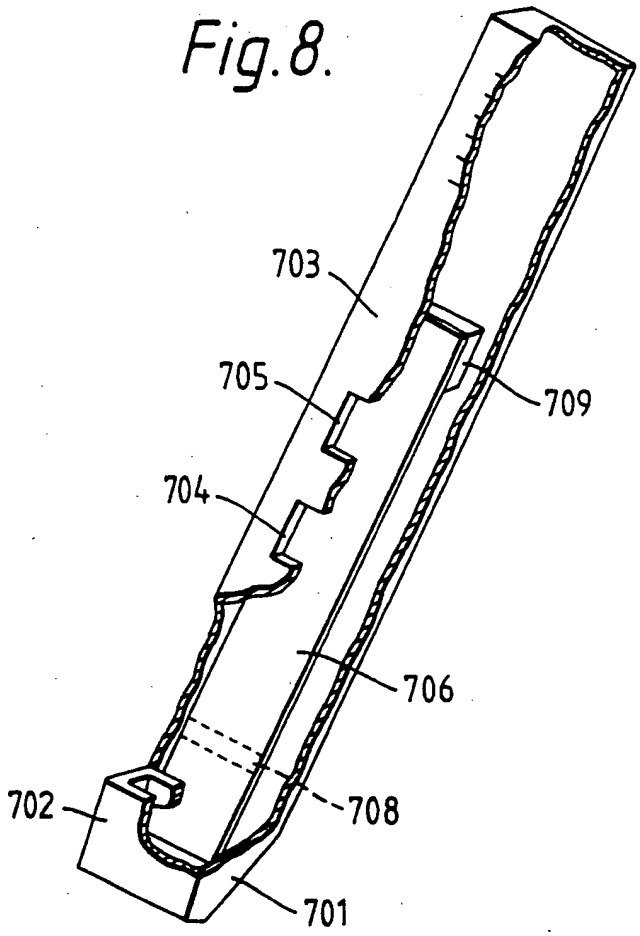
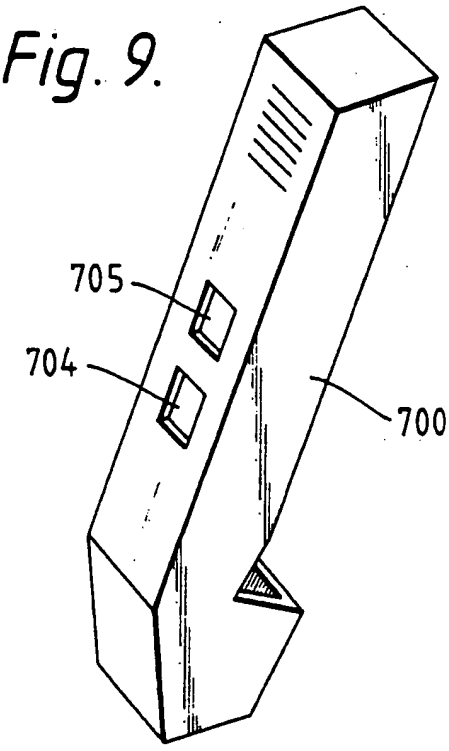


Fig. 9.



I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)*

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 G 01 N 33/558 G 01 N 33/543

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,8505451 (HYBRITECH INC.) 5 December 1985, see page 3, line 5 - page 4, line 31; page 5, line 33 - page 6, line 37; page 8, lines 1-23	1,17,19 ,20,26
X	EP,A,0286371 (SYNTEX (U.S.A.)INC.) 12 October 1988, see column 3, line 26 - column 4, line 37; column 8, lines 9-34; column 12, line 28 - column 13, line 15; column 20, lines 19-46	1,2,7, 12,13, 16,17, 19,20, 23,26
X	EP,A,0302673 (BELTON DICKINSON & CO.) 8 February 1989, see the whole document	1,12,16 ,17,19, 20,23, 24,26
Y		2,7,9

-/-

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18-10-1991	09. 12. 91
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	Nuria TORIBIO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	EP,A,0303784 (MILES INC.) 22 February 1989, see page 7, lines 19-57	2,7,9
Y	WO,A,8808534 (UNILEVER PLC) 3 November 1988, see the whole document, esp. page 11, lines 1-17 (cited in the application)	1,2,7-13,15-26
Y	US,A,4753908 (K. KAWASE et al.) 28 June 1988, see columns 1-5	1,2,7-13,15-26
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A	DE,A,2713963 (C.P. WIELAND) 12 October 1978, see claims; page 5, lines 6-30	1-3,7-11
A	EP,A,0306772 (ABBOTT LABS) 15 March 1989, see the whole document	1,16-26
A	EP,A,0334015 (BECTON DICKINSON & CO.) 27 September 1989	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101079

SA 49136

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 08/11/91
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DE-A- 2713963	12-10-78	None		

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101079 SA 49136

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